Peptide-Induced Self-Assembly of Synthetic Poly(lactide fumarate) Macromer

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Statement of Purpose: Short sequences of amino acids are especially attractive for self-assembly because nanostructures with varying size, shape, morphology, and surface functional group can be assembled by arranging the 24 naturally accruing amino acids in different sequences or by changing the sequence length [38]. It has been demonstrated that the peptide CV6K2 spontaneously self-assembles in aqueous environment to form nanospheres (NPs) with relatively uniform size distribution. Poly(lactide fumarate) (PLAF) macromer alone does not produce NPs but blends of PLAF and amphiphilic poly(lactide-co-ethylene oxide fumarate) (PLEOF) macromers produce NPs with relatively wide distribution. The objective of this work was to investigate self-assembly of PLAF when conjugated with CV6K2 peptide.

Methods: The CV6K2 peptide was synthesized manually on Rink Amide NovaGel resin in the solid phase as described (1). ULMW poly(lactide) (PLA) was synthesized by ring opening polymerization of L-lactide (LA) monomer with diethylene glycol (DEG) as the initiator (2). PLAF was synthesized by condensation polymerization of ULMW PLA with fumaryl chloride (FuCl) (2). Similarly, for PLEOF synthesis, ULMW PLA and PEG were reacted with FuCl (2). CV6K2 peptide was conjugated to PLF macromer by the reaction between the sulfhydryl group of cystine with fumarate group of PLGF. CV6K2-PLAF and PLAF-PLEOF macromers were self-assembled to form NPs by dialysis (2). The morphology of the NPs was examined by TEM. The size distribution of NPs was measured by dynamic light scattering. To measure degradation, NPs were incubated in PBS at 37ºC and the fraction of mass remaining at each time point was determined until complete degradation (NPs not detectable by dynamic light scattering).

Results: The size distribution of CV6K2, PLAF-PLEOF, CV6K2-PLAF, and mutant C(V2K)2V2-PLAF NPs are shown in Figure 1. CV6K2 peptides self-assembled into NPs with 70 nm average size and narrow distribution of 30-110 nm (green curve). PLAF macromers alone did not produce particles but blends of PLAF and PLEOF macromers produced NPs with 300 nm average size and relatively wide distribution of 160-430 nm (blue curve). Interestingly, when CV6K2 was conjugated to PLAF and self-assembled in the absence of PLEOF, NPs with 110 nm average size and relatively narrow distribution of 50-170 nm were produced (red curve). Furthermore, When a mutant C(V2K)2V2 peptide (a peptide sequence with the same composition as CV6K2 that does not self-assemble into NPs) was conjugated to PLAF, the conjugate self-assembled into NPs with average and distribution similar to PLAF-PLEOF blend (light brown curve). These results demonstrate that the CV6K2 peptide induced self-assembly when conjugated to a non self-assembling PLAF macromer. To our knowledge, this is the first time that induction of synthetic polymer self-assembly by peptides has been observed. Figure 2 shows the TEM image of self-assembled CV6K2-PLAF NPs. Figure 3 shows the self-assembled CV6K2-PLAF NPs degrade linearly by matrix erosion.

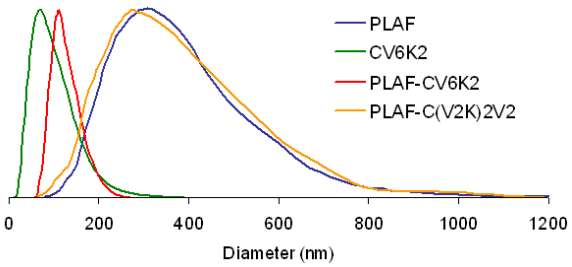

Figure 1. Size distribution of PLAF, CV6K2, CV6K2 conj PLAF, and C(V2K)2V2 conj PLAF NPs.

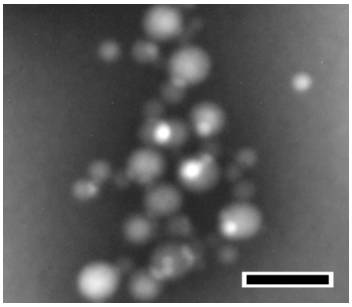

Figure 2. TEM of CV6K2-PLAF NPs (scale bar: 500 nm).

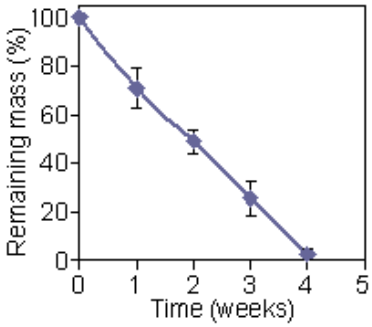

Figure 3. Degradation of CV6K2-PLAF NPs.

Conclusions: PLAF macromer self-assembled into NPs by induction with CV6K2 peptide. To our knowledge, this is the first time that induction of synthetic polymer self-assembly by peptides has been observed.

References

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(2) He X., Ma J., Mercado A.E., Xu W., Jabbari E., Pharm. Res., 25(7):1552-1562 (2008).